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A. J. Malkin, S. Elhadj, M. Plomp

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High-Resolution Spore Coat Architecture and Assembly of *Bacillus* Spores

A.J. Malkin, S. Elhadj and M.Plomp

Physical and Life Science Directorate, Livermore National Laboratory, Livermore, CA, USA

Elucidating the molecular architecture of bacterial and cellular surfaces and its structural dynamics is essential to understanding mechanisms of pathogenesis, immune response, physicochemical interactions, environmental resistance, and provide the means for identifying spore formulation and processing attributes. I will discuss the application of *in vitro* atomic force microscopy (AFM) for studies of high-resolution coat architecture and assembly of several *Bacillus* spore species. We have demonstrated that bacterial spore coat structures are phylogenetically (1-4) and growth medium (5) determined. We have proposed that strikingly different species-dependent coat structures of bacterial spore species are a consequence of sporulation media-dependent nucleation and crystallization mechanisms that regulate the assembly of the outer spore coat (1, 2). Spore coat layers were found to exhibit screw dislocations (3) and two-dimensional nuclei (1-3) typically observed on inorganic and macromolecular crystals. This presents the first case of non-mineral crystal growth patterns being revealed for a biological organism, which provides an unexpected example of nature exploiting fundamental materials science mechanisms for the morphogenetic control of biological ultrastructures. We have discovered and validated, distinctive formulation-specific high-resolution structural spore coat and dimensional signatures of *B. anthracis* spores (Sterne strain) grown in different formulation condition (6). We further demonstrated that measurement of the dimensional characteristics of *B. anthracis* spores provides formulation classification and sample matching with high sensitivity and specificity (6). I will present data on the development of an AFM-based immunolabeling technique for the proteomic mapping of macromolecular structures on the *B. anthracis* surfaces (7). These studies demonstrate that AFM can probe microbial surface architecture, environmental dynamics and the life cycle of bacterial and cellular systems at near-molecular resolution under physiological conditions. This work was performed under the auspices of the U.S. DOE by LLNL under contract number DE-AC52-07NA27344.

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